

# REMOVAL OF SPILLED OIL DEPOSITS THROUGH BIOREMEDIATION

## Abstract

Oil spills have occurred in the environment since the last century, mainly due to transportation and oil processing. Due to the threats, it poses to the environment, the economy, and society, it should be handled carefully. The oil spills can be cleaned up using various physical, chemical, and biological techniques. Bioremediation is the most promising method for pollutant removal compared to the physicochemical method, especially after the Exxon Valdez oil spill. Bioremediation has several advantages over other methods, such as cost-effective and environmentally friendly technology. Both natural and genetically modified microbes can be applied to polluted sites with enriched nutrients. Bioremediation can be divided into bioattenuation, biostimulation and bioaugmentation based on the method followed to remove the pollutants. The researchers have carefully evaluated these methods for oil treatments in the laboratory and, to a lesser extent, in the field.

**Keywords:** Oil Spill; Bioremediation; Microorganisms; Gems.

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## I. INTRODUCTION

Many liquid petroleum hydrocarbons leak into the environment through pipelines, improper waste disposal, storage tanks, etc., causing an oil spill. [1]. It was estimated that 1.7 to 8.8 MMT of oil has been released annually into the world's water [2], of which >90% was directly related to anthropogenic activities. These include equipment and unit cleaning or accidents during transportation [3]. Shipping, onshore and offshore exploration, and transportation account for the majority of petroleum hydrocarbon spills [4].

Oil is a group of related materials rather than a single chemical entity that may be extracted using certain solvents [5,6]. Oil compounds are nonpolar; thus, they are hydrophobic [6]. Under anaerobic conditions, oil hydrolyze into glycerol and long-chain fatty acids [7,8]. The glycerol continues to break down, becoming 1, 3-propanediol [9] and then acetate [10]. Oil-water mixtures with droplet sizes of 150 or less are categorized as free oil, while those with droplet sizes of 20 to 150 are categorized as dispersed oil mixtures. Oil-water mixtures with droplet sizes smaller than 5 microns are categorized as soluble oil mixtures, while oil-water mixtures with droplet sizes smaller than 20 microns are considered emulsified oil mixtures [11].

Oil spills can occur in terrestrial and marine environments and threaten human health and the ecosystem [12]. Oil spills pollute the water, risk explosion and cause fire, ruin the water and air quality, destroy the recreational area, and waste nonrenewable resources. The consequence of oil spills on the natural ecosystem is widespread and long-term. Large-scale marine oil spills, in particular, have drawn much attention due to the catastrophic harm they cause to the ecology. In 1989, as a result of the Exxon Valdez's spill of 37,000 metric tonnes of North Slope crude oil into Prince William Sound, Alaska, thousands of marine mammals and seabirds perished, intertidal and subtidal organisms significantly declined, and there were numerous long-term environmental effects [13]. Minor oil spills from non-point sources, such as urban runoff and boat bilges, pose less risk to the environment and human health. Recent National Water Quality Inventory reports reveal that non-point source pollution continues to be the Nation's leading cause of water quality issues, despite tiny oil spills having historically received significantly less attention [5,14]. Oil spill occurrence is most common in sea and shoreline since the petroleum is usually transported through water. The mutagenic and carcinogenic effects of oil spills in the sea have been proven. Once the oil spill occurs, it prevents oxygen penetration and light diffusion in the bottom layers of the sea [15]. Oil spills in the ocean have a severe adverse effect on marine life and the environment. Therefore, there is a need for a trained workforce who can take suitable action shortly after an oil spill [16].

There are numerous regulations regarding oil spill management, prevention, and compensation. However, oil spills are inevitable, and the government should be ready to respond accordingly. The responsibilities of Governments include creating a national authority, developing a response capability, and developing a federal government plan (NCP) [17].

Physical, chemical, and biological techniques are a few of the typical oil spill countermeasures. Physical removal techniques that are frequently used include tilling, booming and skimming, moving sediment, and water flushing. The main option for cleaning up oil spills in freshwater shorelines and marine habitats of United States is physical

containment and recovery of the free oil. In many countries, chemical techniques, particularly dispersants, have been employed. However, the chemical method has not been extensively used due to its toxicity, ineffectiveness and long-term environmental impacts [5]. The development of less toxic chemical dispersants may increase its potential application.

Although physical and chemical methods are often the first response option in oil spill removal, these methods rarely completely clean up oil spills. Bioremediation is an emerging technology, particularly complete oil spill removal. The bioremediation approach was developed based on the principle that the oil components are readily degradable by microorganisms [18,19]. Bioremediation has several potential advantages over conventional methods, such as being less costly and more environmentally friendly. This article focuses on oil spill removal through bioremediation.

## **II. SOURCES OF OIL**

Oil mills are the main source of oil. The oil content of the oil mill effluents ranges from 4000 to 6000 mg/l [20], with POME being one example. Domestic wastewater that has not been treated has an oil concentration of 50 to 100 mg/l [21]. The largest source of oil in home wastewater is kitchen greywater [22]. Oil-containing effluents are a common byproduct of the food processing industry. Unit cleaning, accidents during transportation and petroleum hydrocarbon spillage during shipping are the major oil source in the ocean [3]. Most petroleum hydrocarbon spillage occurs during shipping, onshore and offshore exploration and transportation [4].

## **III. EFFECTS OF OIL SPILL**

Oil spills all significantly impact the local economy, population, and marine ecosystem. Tourism will be impacted, impairing swimming, sailing, and fishing activities. Freshwater-dependent industries are also impacted and cannot operate again until the water has been cleaned up. The fishing business suffers greatly due to the substantial loss of marine life and the fact that any catch would be covered in oil, rendering it toxic and inedible. Human exposure to oil spills may negatively affect the nervous system, causing acute toxic effects, eye and skin irritation, and the respiratory system. People who lived in the affected areas experienced headaches, migraines, and nausea. Consuming seafood that has been exposed to oil is hazardous because oil products include polycyclic aromatic hydrocarbons (PAH). These can cause cancer in people. After an oil spill, seagrasses and other vegetation are lost in the marine environment. Because most marine animals eat these, it is dangerous. Living coral is also vulnerable to oil slicks. When coral dies, wave erosion may wash away the coral reef. This results in the habitat loss of several fish and animals.

Mangroves are salt-tolerant trees that offer homes to sea creatures, including seabirds, crabs, oysters, and fish breeding grounds. Their extensive root systems stop soil erosion. Mangroves are vulnerable to oil spills because they obtain oxygen through lenticels on aerial roots, also known as pneumatophores, which may become clogged by oil and stop the oxygen supply. Toxic components in oil may also upset plants' mechanisms of maintaining salt balance. Seashore species like sea turtles lose their native environment due to oil spills. Sea turtles can be exposed to oil spills as they stay ashore for the nest, and when the oil floats over to beaches, it destroys the natural habitat of seaside animals like sea turtles. Sea birds are

affected by oil spill as it swims and dives into the water for their food and are most likely to be damaged. Even a small amount of oil can cause danger to the bird. The oil coating over the birds does not allow them to fly and thus destroys their natural waterproofing. Many seabirds have wettable feathers that need to be carefully dried before flying. Seabirds that come into contact with oil on their feathers swallow the oil as they attempt to preen. Clams and oysters are examples of filter feeders that take in surface water through their gills and filter it to remove any food. According to accounts, the Exxon Valdez oil disaster killed between 2,50,000 and 5,00,000 seabirds [23]. The oil in the water gets concentrated within shellfish and then accumulates in their predators in a higher concentration; accordingly, the entire food chain will accumulate oil residues.

Smothering, in which oil covers the outside of a mammal's body, covering fur and making it difficult for the animal to breathe, is one of the physically frightening processes that happen to mammals. Animals, their prey, the air they breathe, and the hair they clean can all contain toxic substances from the spilled oil, with both immediate and long-term harmful effects. The marine fish are also at grave risk from the oil spill, which results in their deaths due to the heavy oil exposure.

#### IV. OIL SPILL REMOVAL STRATEGIES

The faster response to the spill prevents the spread and contamination of oil in the environment [3]. Controlling the source of the spill and stopping the oil spread is the first crucial action after an oil spill. Any plan or technique can be used to contain the spill and any unfavorable effects. Several methods and treatments are available to countermeasure the oil spill, including physical, chemical, and biological methods [24]. The physical method includes booms, barriers and sorbents, dispersants, skimmers and *in situ* burning [17]. The common strategy used for controlling the oil spread with barrier application and concentrating the oil into a thick layer by booms to enable the oil removal by different skimmers. The water is often filtered in the skimmer boats and sent back to the ocean while the oil is transferred to a holding tank. *In situ* burning is another method that is followed to remove the oil contaminant from the ocean. After *in situ* burning, it is necessary to perform a toxicity assessment. This approach was followed in the Deepwater Horizon accident [23].

Dispersants are used in the chemical method, which is sprayed on the oil spill to break it up into small droplets to make it consumable by microorganisms more efficiently [23]. Typical dispersants used for oil spill removal are Sulfonated naphthalene formaldehyde condensate (SNFC), sulfonated melamine formaldehyde condensate (SMFC), lignosulfonates, polyacrylamides.

Conventional methods can effectively remove oil spills but produce several hazardous compounds [24]. In contrast, biological methods detoxify hazardous compounds and are less disruptive than excavation methods in the case of soil.

#### V. BIOREMEDIATION OF OIL SPILLS

Bioremediation aims to enhance the metabolic activity of microorganisms in the polluted sites and, consequently, stimulate the oxidation-reduction of the oil contaminants. In this method, microbes degrade the organic compounds of the contaminants [25]. This method

was developed in the 1940s and gained more attention after the Exxon Valdez oil spill in the 1980s [26]. It requires a longer time for effective cleanup. Bioremediation is cost-effective, has no significant adverse effects, has simple technology and minimal physical disruption [27, 12]. The main disadvantage of this method is that a specific approach is needed for each polluted site and each type of spill. Microbes use petroleum hydrocarbon as a substrate to decompose pollutants into water, CO<sub>2</sub>, and other harmless compounds [28]. When bioremediation is used to treat an oil spill, some materials are added to the polluted environment. The materials may be the nutrients to enhance the growth of indigenous microbes or non-native microbes having enhanced the ability for hydrocarbon degradation were added to the contaminated environment. It is used as a complementary treatment after conventional cleanup [27]. It requires a longer period for effective cleanup, and bioremediation is less effective in a highly contaminated environment [29]. Bioremediation is effective and faster in the case of a shoreline oil spill. In the case of burned oil, materials such as microorganisms and nutrients should be added to enhance the cleanup [30].

The adaptation skills of the microbes and their resistance in the polluting environment is also an important factor [15]. However, even adapted microbes are ineffective for bioremediation in a highly polluted environment [26]. Because during oil spills, the level of petroleum hydrocarbons goes beyond the tolerable limit of the microbes [31].

Different microorganisms such as bacteria, fungi, microalgae and yeast can degrade hydrocarbon pollutants. Bioremediation can be performed in two ways; i) *in situ* and ii) *ex situ* [32]. In the *ex-situ* method, the contaminant matrix was extracted and treated elsewhere, while during *in situ* process, bioremediation occurs in the place of contamination [25]. The *in-situ* approach is cost-effective and much safer than *ex-situ* [32].

## VI. ESSENTIAL FACTORS FOR MICROBIAL BIOREMEDIATION

Bioremediation is greatly influenced by a number of factors, including physical and chemical characteristics. Temperature, pressure, and pollutant surface area are physical characteristics. Nutrient and oxygen availability, salinity, acidity, and pollutant type and composition are chemical parameters. Most of the variables can be changed to speed up natural bioremediation, but some factors, like salinity, cannot be changed in the field [27].

Temperature is the major factor that impacts viscosity, solubility and toxicity. As the temperature rises, biodegradation accelerates. In ocean, freshwater, and soil, the ideal temperatures for biodegradation were 15-20 °C, 20-30 °C, and 30-40 °C, respectively. Additionally necessary for the oxidation and destruction of chemical pollutants is dissolved oxygen. In the sea and lakes, oxygen levels are often unrestricted. However, some wetlands and sediments may lack oxygen. In such cases, anaerobic degradation can be carried out. Upflow anaerobic sludge blanket (USAB) is the bioreactor system used in *ex-situ* bioremediation. The advantage of anaerobic degradation over aerobic is less space utilization and less energy requirements [33]. Pressure is also an essential factor that can impact bioremediation. When the pressure increases, the rate of degradation decreases. The surface area of the pollutant can impact the oil and water interface. The rate of bioremediation increases with increasing surface area. At higher pH, the rate of petroleum hydrocarbon degradation increases [27].

## VII. TYPES OF BIOREMEDIATIONS

Bioremediation can be divided into three types based on the method followed to remediate the pollution. They are bioattenuation, biostimulation and bioaugmentation. The bioattenuation (natural attenuation) process is based on the metabolic potential of microorganisms to detoxify or transform the contaminants. Although it is frequently regarded as a do-nothing approach, it necessitates ongoing monitoring of contamination sites. The exact pollutant and site conditions determine how long natural attenuation will take. Natural attenuation in some sites may take a long time because of its persistent nature and lack of appropriate degrading microorganisms.

The right environmental conditions for microorganisms can be created to promote biodegradation. This process is known as biostimulation. There are two ways by which biodegradation can be stimulated; they are 1. supplying fertilizers (nitrogen and phosphorus), growth supplements and trace minerals; 2. providing environmental requirements like pH, temperature and oxygen to speed up their metabolism rate and pathway. All these actions are taken to hasten the growth and activity of oil degraders. This strategy is often referred to as nutrient enrichment or fertilization [27]. With the addition of fertilizers like iron, phosphorus, and nitrogen, the biodegradation rate increased three to five times, yielding positive findings on sediments of the Alaskan coast damaged by the Exxon Valdez spill. It was shown that adding nitrate to saltwater is more successful at degrading crude oil than adding ammonia, although adding ammonia to salt-marsh soil is more effective [27].

In bioaugmentation, the polluted site is infused with microorganisms that have an improved capacity to break down petroleum hydrocarbons. Few hydrocarbon-degrading microorganisms are present, or when a specific hydrocarbon must be destroyed that cannot be done by native bacteria (such as polyaromatic hydrocarbon), this method is used. The degradation of hydrocarbons is known to occur in 70 genera of microorganisms. The target site is frequently supplemented with non-native microorganisms from other contaminated settings [27]. Alternatively, bacteria from the target site are isolated, mass cultivated in bioreactors in a lab setting, and then introduced to the target site as an inoculum. This procedure, known as autochthonous bioaugmentation, allows the local bacteria of the contaminated site to do bioremediation [26]. The adaption issue is avoided when the seeding is carried out by improved native organisms collected from the target place.

## VIII. MICROORGANISMS FOR BIOREMEDIATION OF OIL SPILLS

According to reports, more than 200 different types of bacteria, fungi, and yeasts may break down petroleum hydrocarbons. These species were ordinarily discovered in soil, freshwater, and marine settings. Petroleum hydrocarbons could be broken down by about 79 bacteria, 9 cyanobacteria, 103 fungi, 14 microalgae, and 56 yeasts [34, 27]. Polycyclic aromatic hydrocarbons (PAHs) can be broken down by naturally occurring soil bacteria, such as *Pseudomonas* strains found in soil and aquifers [4]. Other microbial species with the ability to degrade petroleum hydrocarbons are *Alcaligenes* sp., *Alcanivorax* sp., *Acinetobacter* sp., *Bacillus* sp., *Capnocytophaga* sp., *Cellulomonas* sp., *Corynebacterium* sp., *Dietzia* sp., *Enterobacter* sp., *Flavobacter* sp., *Gordonia* sp., *Microbulbifer* sp., *Micrococcus* sp., *Moraxella* sp., *Providencia* sp., *Roseomonas* sp., *Sphingobacterium* sp., *Sphingomonas* sp., *Stenotrophomonas* sp., *Streptococcus* sp., and *Yokenella* sp. [24].

Some fungi are also able to degrade pollutants. However, they require a longer time to degrade petroleum hydrocarbons effectively. Fungi belonging to *Amorphoteca* sp., *Penicillium* sp., *Aspergillus* sp., *Graphium* sp., *Fusarium* sp., *Talaromyces* sp., *Neosartorya* sp., and *Paecilomyces* sp. are capable of degrading petroleum hydrocarbons [35]. Yeast species, including *Candida* sp., *Pichia* sp., and *Yarrowia* sp., can also degrade oil compounds in the contaminants [24]. Some reports suggest fungi can degrade petroleum better than bacteria in certain circumstances. Still, there is little information on fungal bioremediation in polluted marine sites [15].

Long-term exposure of microorganisms to a polluted environment leads to genetic selection. After adaptation, the microbes develop hydrocarbon catabolic genes in the plasmid and degrade hydrocarbons [32]. Microbes with increased oil degradation ability were observed in *Alcanivorax* sp. and *Cycloclasticus pugetii* [34]. Using indigenous microorganisms available in the contaminated sites is most appropriate for bioremediation as the indigenous microorganisms adapted quickly to the available environmental conditions. The slow decomposition rate and toxicity of some organic pollutants is the fundamental drawback of using native microorganisms for bioremediation. This is severe in new man-made pollutants since the microbes have not developed any resistance mechanism for their degradation [36]. When the indigenous microbes failed to degrade hydrocarbons, oil-degrading microorganisms were added to the contaminated environment [27].

## IX. GENETICALLY MODIFIED MICROORGANISMS FOR BIOREMEDIATION OF OIL

The first genetically modified microorganism (GEM) was developed in the 1970s. These microbes were named "superbugs" and can degrade oil [37]. Superbug was built in 1979 by Ananda Mohan Chakrabarty. The US government granted a patent for the construction and use of superbug. In 1990, the American Government allowed to use the superbug to clean up oil spills in Texas state water. Superbug is a multi-plasmid strain containing CAM-OCT-XYL-NAH plasmids capable of degrading camphor, octane, hexane, decane xylene, toluene and naphthalene. The construction of a superbug involves the following steps: 1. Identification and isolation of parent strains (*P. putida*) that could degrade camphor, octane, xylene and naphthalene from polluted soils; 2. conjugative transfer of plasmids; 3. selection of superbug from this mixture of strains done by culturing the strains in the presence of all the four pollutants. Only superbugs can survive in that medium and it is subcultured in fresh medium lacking the pollutants for future use; 4. mass culture of superbug is done by either culturing in a suitable liquid medium in a large bioreactor or sprinkling over paddy straw and the straw is dried in the shade. The bacteria-inoculated straw can be stored for over a year to treat oil spills.

Early in the 1980s, as genetic engineering techniques advanced, the development of GEM gained in popularity. Two GEM strains were created and patented in 1981. *Pseudomonas putida* (NRRL B-5473) and *Pseudomonas aeruginosa* (NRRL B-5472) are the two. These strains have the genes to produce the crucial proteins needed to break down naphthalene, camphor, and salicylate [38]. These strains contain two operons (xylIUCMABN and xylXYZLTEGFJQKIHSR) that encode the m-ethyl toluene, m-, and p-xylene metabolism-related enzymes. The first GEM that was authorized for use in bioremediation in an actual field was *P. fluorescens* HK44. This study was done to aim for

long-term bioremediation of naphthalene-contaminated environment. The plasmid, pUTK21, present in this bacteria, was created by introducing the transposon Tn4431 into the NAH7 plasmid obtained from *P. fluorescens* 5R. The genes that support the route for naphthalene decomposition and the gene cassette (*lux*) were responsible for the simultaneous degradation of naphthalene and the luminous signal [38].

During the Deepwater Horizon oil leak in 2010, researchers at the University of Texas at Austin discovered the genetic code of petroleum hydrocarbon breakdown. They have sequenced the DNA of microbes that can degrade oil in order to learn more about the genetic makeup of various bacterial species. Since the degradative mechanism, the enzymes, and the essential genes are known and biochemical reactions are clearly explained, and it is possible to build GEMs with improved capacity for the biodegradation of organic molecules. The ability of some bacteria to degrade oil was found to be much more than anticipated, especially for aromatic hydrocarbons (for instance, *Alcanivorax* was once thought to be incapable of degrading oil) [38].

For the proper development of GEM, there is a need to understand the breakdown mechanism of petroleum hydrocarbons, biochemical pathways, operon arrangement and genetic basis of interaction [37]. The main drawbacks of GEM are their ability to survive in the environment and public acceptance, which prevents their widespread use in bioremediation [27]. Other issues with GEMs include the potential for gene transfer to other microorganisms and the co-release of antibiotic resistance markers, which restricts research on using GEMs in practical fields and raises concerns about the environment and public health safety.

Due to GEMs' negative environmental effects, like gene transmission, several authorities are reluctant to permit their discharge. However, it should be highlighted that GEMs are obtained from another microbe and do not introduce new genes into the environment. As a result, the introduced designed microorganism typically does not survive for an extended period of time after exhausting its particular substrate. The following techniques can be used to reduce the possible risk of GEMs in the real world: 1. Employing genetic barriers to prevent recombinant microorganisms from surviving and transferring genes in the environment. A novel tactic is the creation of suicidal GEMs, which may be accomplished by adding antisense RNA and a degradative operon to the construct. The restriction can be performed by using transposons empty of transposase genes or by removing conjugation genes from a plasmid [38]. These innovative GEMs reduce the harm bacteria provide to humans and the environment by making them sensitive to death after completing the breakdown of pollutants.

The bioremediation of polychlorinated biphenyl-polluted plants was a successful use of GEM. In this instance, the biphenyl dioxygenase enzyme of *Pseudomonas alcaligenes* KF707 and *Pseudomonas* sp. LB400 was altered by altering its substrate specificity. Combining their spectrum of available substrates increased the ability of microorganisms to oxidize double ortho- and double para-substituted PCBs [39].



## X. CONCLUSION

Oil spill occurrence in land and water is not a new problem. The spill occurring in soil or water greatly threatens the natural ecosystem, flora and fauna. Compared with the physicochemical method of pollutant removal, biodegradation is a more effective and eco-friendly method without disrupting the natural environment. Although numerous researchers have researched this method, a high hydrocarbon removal rate was found in the lab, and the right technology for field applications has yet to be created. A contemporary method called bioremediation can be used with other physical and chemical processes to remove various environmental pollutants. Since it is a sustainable method of managing environmental contamination, additional study in this field is required. Most of the research into GEMs' potential for bioremediation was conducted in lab settings. However, long-term bioremediation in the actual field is necessary to determine the true impact of GEMs. This is required to assess the ecosystem's overall efficacy and potential risks.

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